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Original Article

Effect of cooking on functional properties of germinated black glutinous rice (KKU-ULR012)♣

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Abstract

The aim of this research was to investigate the changes in functional properties of germinated black glutinous rice (KKU-ULR012) after cooking. Black glutinous rice grains were obtained from Faculty of Agriculture, Khon Kaen University, Thailand. The rough grains were soaked for 12 hrs, then germinated for 30 hrs at 35±2°C (95%RH), dried at 45±2°C for 8 hrs, dehusked and cooked either using a microwave oven or a pressure cooker. The cooked grains were dehydrated in two stages, 85±2°C for 1 hr and 45±2°C for 12 hrs until the final moisture content was 10±2%$_{wb}$. The antioxidant activity, anthocyanins, GABA and γ-oryzanol contents, and the microstructure of the dehydrated grains were then characterized. Germination process induced a 2.55 fold increase in GABA content compared to non-germinated KKU-ULR012. The germinated KKU-ULR012 gave DPPH value, anthocyanins and γ-oryzanol contents of 33.74±0.15 mgTrolox/100g$_{db}$, 182.89±0.48 mg/100g$_{db}$ and 37.72±0.16 mg/100g$_{db}$, respectively. Anthocyanins in cooked germinated KKU-ULR012 diminished almost 88-89% after cooking. The cooking methods employed strongly influenced the antioxidant activity and anthocyanins content that the pressure cooking tended to prevent loss of anthocyanin content and antioxidant activity. The GABA, γ-oryzanol and anthocyanins contents and antioxidant activity of germinated grains cooked in the pressure cooker were higher than the samples cooked in the microwave oven ($p<0.05$). For pressure cooking, the cooked grains gave DPPH, ABTS, anthocyanins and γ-oryzanol contents of 9.89±0.35 mgTrolox/100g$_{db}$, 1.79±0.04 mgTrolox/100g$_{db}$, 21.60±0.14 mg/100g$_{db}$ and 37.16±0.70 mg/100g$_{db}$, respectively. The rice grains cooked by pressure cooking were more moist and sticky than the grains cooked by microwave cooking. The microstructure examined by SEM showed that the center of the dehydrated cooked rice grain was smooth indicating starch gelatinization whereas the surface revealed fission of the grain. This study found that germinated KKU-ULR012 cooked by the pressure method can be one of the native rice varieties that possess a potent source to enhance GABA and γ-oryzanol contents as well as antioxidant activity.

Keywords: black glutinous rice, germination, cooking, antioxidant activity, γ-oryzanol

1. Introduction

Pigmented rice has been reported as potent and a viable sources of antioxidants for functional foods, especially γ-oryzanol and anthocyanins in the pericarp (Yawadio *et al.*, 2007). Positive health effects of the pigments in the bran layer of rice have been reported, particularly in germinated pigmented rice. Brown rice or rough rice can be germinated by soaking in warm water for 24 hrs. The process of germination induces γ-aminobutyric acid (GABA) in rice grains (Moongnarm and Saetung, 2010). Manufacturers claim that products containing GABA can help boost the brain's GABA levels and, in turn, treat anxiety, stress, depression, and sleep problems (Pei-Ni *et al.*, 2005). Rice is heated along with water,

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which is absorbed by the rice as cooking proceeds. Cooking of rice can also be described as the degree of starch gelatinization present in rice (Juliano, 1985). Gelatinization occurs over a range of temperatures and can commence anywhere between 55 and 80°C depending on the rice variety (Bhattacharya, 1979). The degree of the starch gelatinization (%) increased with increasing temperature (Ahromrit *et al.*, 2007). The degree of gelatinization of fully cooked rice was in the range of 90–100% measuring by pressing method (Daomukda *et al.*, 2011). There are two common rice cooking methods, namely, boiling method and electric rice cooking. A number of reports have revealed that thermal processes, i.e., pressure-heating and microwave heating could reduce cooking time and is becoming increasingly popular and important in rice cooking (Gould and Golledge, 1989; Giuliani *et al.*, 2010). Pressure cooking has been proposed to inactivate micro-organism, remove aflatoxin (78-88%) and minimal nutrient changes in cooked rice (Kouniaki *et al.*, 2004; Park *et al.*, 2005; Park and Kim, 2006). Daglioglu *et al.* (2000) reported that microwave energy eects on various food components could dier signicantly from those of conventional cooking. Microwave heating impacted significant changes in viscosity properties of both waxy and non-waxy rice starches (Anderson and Guraya, 2006). In the last decade, helpful reviews by Nicoli *et al.* (1999), Klein and Kurilich (2000), and Kaur and Kapoor (2001) provide brief information on the antioxidant activity as influenced by processing. Baking of purple wheat bran at 177°C for 20 min has not altered the total phenolic content in the processed samples (Li *et al.*, 2007). By contrast, the total phenolics and total antioxidant activity of sweet corn has increased by 54 and 44%, respectively, after thermal processing at 100–121°C for 10–50 min (Dewanto *et al.*, 2002). Pitiwiwattanakul *et al.* (2011) reported that a quick-cooking process by heat treatment using water, steam and dry caused a reduction antioxidant properties and polyphenol content in germinated rice. The effect of cooking methods on antioxidant activity is not the same among the food products. Use of pressure or microwave heating to cook germinated black glutinous rice (KKU-ULR012) may affect the rice functionalities. This research aimed to study the effect of two cooking methods, microwave and pressuring methods, on the texture properties of germinated black glutinous rice. Further, anthocyanins, GABA and g-oryzanol contents, antioxidant activity and microstructure of cooked-germinated KKU-ULR012 after dehydration were also determined.

2. Materials and Methods

2.1 Materials

Rough rice of *Oryza sativa* L., cultivar KKU-ULR012 (a black glutinous rice cultivar growing in the Northeast of Thailand) was obtained from the Faculty of Agriculture, Khon Kaen University. Samples stored at 4±1°C. Prior to germinating the rough rice was taken out from a refrigerator to attain room temperature for 1 day. The moisture content of rough rice was determined by drying a sample at 105±1°C for 24 hrs in a hot air oven (Memmert, model U30, Schwabach, Germany) according to AACC (2000).

2.2 Preparation of germinated rough rice

Germination process was conducted by the method described by Moongngarm and Saetung (2010), and the conditions employed were the same as Sutharut and Sudarat (2012). Rough rice (5 kg) was soaked in RO water (at a ratio of rice to water of 1:1.5) at 35±2°C for 12 hrs. The steeping water was changed every 6 hr and drained at the end of soaking. The steeped rough rice kernels were distributed on double layers of cotton cloth and placed in plastic basket. This basket was then covered by double layers of cotton cloth. The germination occurred in a germinating chamber maintained at 35±2°C for 30 hrs. The germinated seeds were dried at 45±2°C, to approximately 9-10% moisture content. The husk was removed from the germinated kernel using a laboratory de-husker, in order to obtain germinated black glutinous rice. 1 kg samples of germinated black glutinous rice were vacuum-packed in Nylon/LDPE bag, and stored at 4±2°C, until further experiments.

2.3 Preparation of cooked germinated rice

For each experiment 200 g of germinated black glutinous rice was washed thoroughly in water at ambient temperature in order to clean and remove dust particles. In the case of microwave cooking, the germinated black glutinous rice was soaked in vacuum oven at 35±1°C for 1hr (at a ratio of rice to water of 1:1.2). The moisture content of steeped rice was in the range of 30-35%$_{db}$, which is sufficient for gelatinization (Karunarathna *et al.*, 2010). The germinated black glutinous rice was cooked in a microwave oven (LG, model MS2427BW) of a rated power of 800 Watt for 30 min, followed by a 10 min post-cooking period. For a pressure cooker (All American, model 1941X), it operated at around 15 pounds/sq. inch, the pressure built up inside the cooker, after an initial emission of steam for 30 min (at a ratio of rice to water of 1:1.2, w/w). The cooked grains were pressed between two glass plates. No opaque core was observed when starch granule fully gelatinized (90–100%). Texture properties of cooked germinated black glutinous rice were determined. After complete cooking the sample obtained from the rice holder was frozen at -18±1°C for 4 hrs and thawed at room temperature (30±1°C) for 1 hr (modificated after Luh, 1991). The samples cooked by microwave and pressure cooking were then dried in a cabinet tray dryer at 85±1°C for 1 hr and 45±1°C, to give approximately 9-10% moisture content (Rewthong *et al.*, 2011).Treated samples were determined antioxidant activity, anthocyanins, GABA and γ-oryzanol contents, and the microstructure.

2.4 Determination of antioxidant activity

2.4.1 DPPH radical scavenging activity assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts was measured according to the modified method of Brand-Williams *et al.* (1995). The reaction mixture contained 3 ml DPPH working solution (4.73 mg of DPPH in 100 ml ethanol) to which was added 100 µl rice extract. The mixture was shaken and held for 30 min in the dark at room temperature (30±1°C). The absorbance was then read at 515 nm using a UV-visible spectrophotometer (Cecil, Aquarius 7400). The inhibition percentage of the absorbance of the DPPH solution was calculated using the following equation:

$$\text{Inhibition \%} = [(A_{blank} - A_{sample}) \times 100] / A_{blank} \quad (1)$$

where, A_{blank} is absorbance of control blank, and A_{sample} is absorbance of sample extract. Trolox was used as standard to convert the inhibition capability of the extract solution to the Trolox equivalent antioxidant activity. The results were expressed Trolox equivalent (mg/g_{db}) of sample.

2.4.2 Trolox equivalent antioxidant capacity (TEAC) assay

The ABTS radical cation scavenging assay was analyzed following a modified method of Re *et al.* (1999). A stable stock solution of ABTS radical cation was produced by reacting a 7 mM aqueous solution of ABTS with potassium persulfate in a dark at room temperature for 12–16 hrs before use. Rice extract (10 µl) was allowed to react with 4.5 ml of a diluted ABTS radical cation solution (absorbance of 0.70±0.05 AU at 734 nm). The absorbance was then read at 734 nm using a UV-visible spectrophotometer (Lambda 25, Perkin Elmer, U.S.A.). Results were expressed as Trolox equivalents antioxidant capacity (TEAC) in mg of Trolox per g of flour.

2.5 Determination of total monomeric anthocyanin pigment content

Total monomeric anthocyanin pigment content of the rice samples was determined, according to the modified pH differential methods of Giusti and Wrolstad (2000) and Hosseinian *et al.* (2008). Briefly, 100 µl of the sample extract was mixed thoroughly with 5 mL of pH 1.0 potassium chloride buffer. The mix was vortexed and then allowed to stand for 15 min. The absorbance was then measured at 515 and 700 nm against distilled water in a UV–visible spectrophotometer (Lambda 25, Perkin Elmer, U.S.A.).The extract was also mixed similarly with pH 4.5 sodium acetate buffer, and the absorbance was measured at the same wavelength after standing for 15 min.

$$\text{Total anthocyanin content (mg/L)} = (A \times MW \times DF \times 1000) / \varepsilon \times l \quad (2)$$

where A = [(A515–A700) pH 1.0 – (A515–A700) at pH 4.5], MW is equal to 449.2 (molecular weight of cyanidin-3-glucoside),DF is the dilution factor of sample, and ε is the molar absorptivity of cyanidin-3-glucosideequal to 26,900.

2.6 Determination of γ-aminobutyric acid

The extraction and determination of γ-aminobutyric acid were performed, according to the modified method of Ohtsubo *et al.* (2005) and Ratahakrut et al. (2007). The ground rice sample (2.5 g) was mixed with 25 ml 70% ethanol, and agitated in a vortex mixer for 10 min. The mixture was centrifuged for 15 min at 12,000 g (at 4°C). Three extraction replications were used for each sample. The extracts of rice were analyzed by HPLC. The system consisted of an HPLC (Waters 2690 Alliance, U.S.A.) connected to fluorescent detectors, excitation wavelength 270 nm and emission wavelength 315 nm. The sample (10 µl) and a gradient mobile phase (1.0 ml/min) was injected into a water symmetry reverse-phase analytical column (C18, 150 x 3.9 mm, 5 µm) at 40°C. The initial mobile phase conditions were Trifluoroacetic acid (TFA) (0.05%), acetonitrile (100%) and methanol (100%).

2.7 Determination of γ-oryzanol analysis

The extraction and determination of γ-oryzanol were performed, according to the modified method of Chen and Bergman (2005) and Imsanguan *et al.* (2008). The ground rice sample (3 g) was mixed with 30 ml methanol and shaken using a vortex mixer for 10 min. The mixture was centrifuged for 15 min at 12,000 g (at 4°C). Three extraction replications were used for each sample. The extracts of rice were analyzed by HPLC. The system consisted of an HPLC (Waters 2690 Alliance, U.S.A.) connected to UV detectors (γ-oryzanolwas detected at UV wavelength 325 nm). A water symmetry analytical column (C18, 150 x 3.9 mm, 5 µm) and a gradient mobile phase (1.0 ml/min) were used, in order to separate the compounds of interest. The initial mobile phase conditions were acetonitrile (50%) and methanol (50%). The total HPLC run time was 23 min.

2.8 Textural properties of cooked-germinated rice

The textural properties of samplescooked by microwave and pressure cooking were measured by Texture Analyzer (TA.XT Plus, UK). The cooked samples comprising about 30 g were placed in a cylinder holder. The cylindrical probe with a diameter of 50 mm was used to compress the kernels to 85% deformation at a pre-test speed of 1 mm/s and post-test speed of 10 mm/s. The resulting force-deformation data were analyzed and the average values of hardness and

stickiness were calculated. The hardness and stickiness of the sample were defined as the maximum force on the first compression and the negative force of the first cycle (during the pulling out of the cylindrical probe), respectively.

2.9 Scanning electron microscopy

The microstructure of cooked-germinated KKU-ULR012 after dehydration was observed using a scanning electron microscopy (LEO 1450VP, Germany) at 10 kV. The cross section surface of the grains was also observed. Samples were attached to a SEM stub using a double-backed cellophane tape. The stub and sample were coated with gold (Sutter coater sc7620), and then examined and photographed.

2.10 Statistical analysis

All data were subjected to the analysis of variance (ANOVA) using SPSS software and are presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests at a confidence level of 95%. All experiments were performed in triplicate.

3. Results and Discussion

3.1 Effect of cooking methods on antioxidant activity, anthocyanins, GABA, and γ-oryzanol content

The uncooked-germinated KKU-ULR012 gave DPPH and ABTS values, GABA, anthocyanins and γ-oryzanol contents of 33.74±0.15 mgTrolox/100g_{db}, 8.00±0.05 mgTrolox/100g_{db}, 10.84 ± 0.31 g/100g_{db}, 182.89±0.48 mg/100g_{db} and 37.72±0.16 mg/100g_{db}, respectively. Germination process induced a 2.55 fold increase in GABA content compared to non-germinated grains (data not shown). When germinated rice grain was cooked by a pressure cooker, the cooked-germinated KKU-ULR012 had higher anthocyanins, γ-oryzanol and GABA contents, DPPH and ABTS values, than the samples cooked in a microwave oven ($p \leq 0.05$) (Figure 1). Moreover, the results indicated that both microwave and pressure cooking affected the reduction in anthocyanins

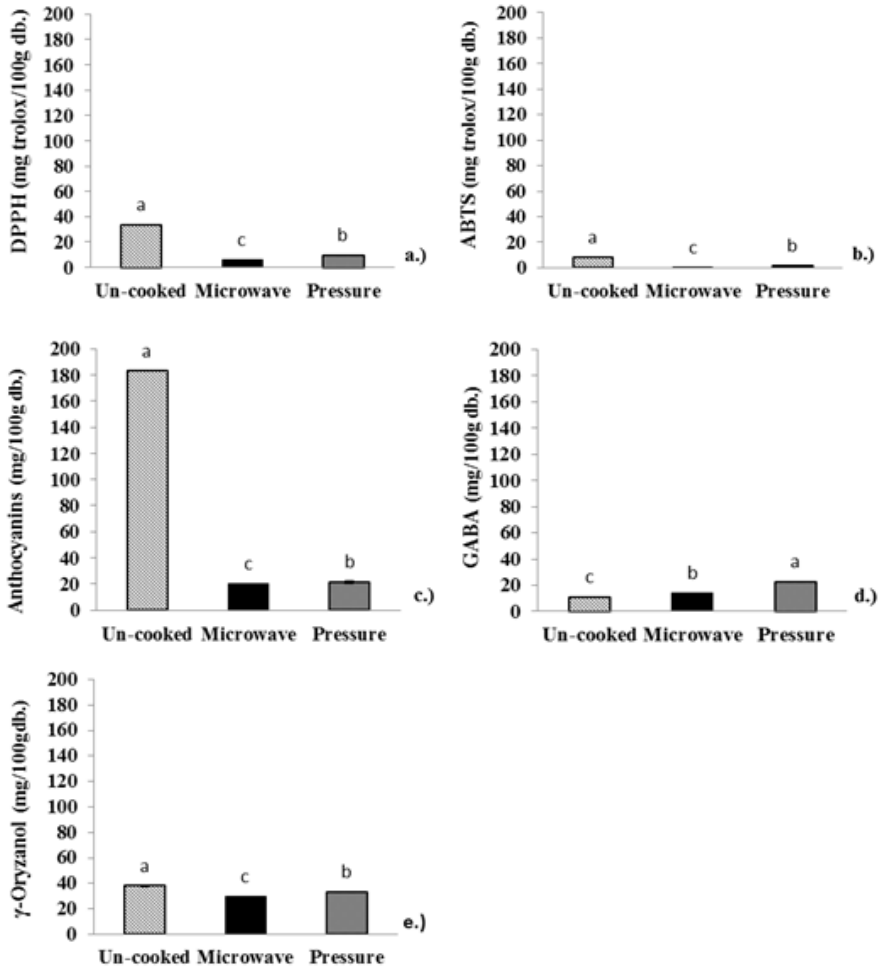

Figure 1. DPPH (a.), ABTS (b.), anthocyanins (c.), GABA (d.) and γ-oryzanol (e.) contents of cooked rice (microwave and pressure cooking) and un-cooked germinated black glutinous rice (KKU-ULR012).

(pd"0.05) due to water soluble compounds and heat sensitivity; however, cooking process retained the GABA content due to the heat stability. Anthocyanins in germinated KKU-ULR012 diminished almost 88-89% after cooking. Among the heat induced fully cooked-germinated KKU-ULR012, the pressure cooker heating is a better method than the microwave heating in term of large holding for functional properties. Pressure cooker puts pressure on the rice grains being cooked, increasing the boiling point of the water inside. Thus, the water can be heated to a much higher temperature before boiling, which can hasten the cooking time as superheated water only coats the grains instead of escaping like water vapor does. While germinated KKU-ULR012 was heated up by microwave cooking, the rice grains released water vapor and permitted more rapid heat penetration, heating these rice grains even more with the boiling temperatures, eventually causing the signicant reduction of antioxidant activities and anthocyanins content in the cooked-germinated KKU-ULR012. Alajaji and El-Adawy (2006) reported that boiling, autoclaving (pressuring) and microwave cooking affect the composition, anti-nutritional factors, and nutritional quality of chickpeas. However, microwave cooking caused slight losses in B-vitamins and minerals, while boiling and autoclaving caused signicant losses. In addition, many researchers have reported that pressure treatment can induce proteolysis to produce free amino acids, and g-aminobutyric acid (GABA) (Kinefuchi *et al.*, 1999; Ahmed *et al.*, 2007; Shigematsu *et al.*, 2010). Anthocyanins, and γ-oryzanolare related to the antioxidant activity of pigment rice grain (Patel and Naik, 2004). Both germinated KKU-ULR012 cooked by microwave and pressure methods present antioxidant potential and nutritional function of GABA.

3.2 Textural properties of cooked-germinated KKU-ULR012

Textural properties were presented in term of hardness and stickiness (Figure 2). The textural characteristics of cooked-germinated KKU-ULR012, rice grains showed that hardness values decreased but stickiness values increased compared to the non-germinated black glutinous rice grains (pd"0.05) cooked by the same methods. Germinated KKU-ULR012 cooked by pressure reduced the hardness values more than samples cooked by microwave (shown in Figure 2a). The pressure cooked samples also had stickiness values more than microwave cooked samples (shown in Figure 2b).This may be due to the better gelatinization and more fluidity of rice starch structure in the pressured samples. These results were similar to those reported (Lyon *et al.*, 2000; Daomukda *et al.*, 2011). Zhou *et al.* (2007) reported that stickiness and hardness are related to the hydration process of starch granules. During cooking, rice granules absorb moisture and swell to a great extent compared to their initial size. The granule expansion causes ruptures and amylose leaching (Tester and Morrison, 1990). The leaching component scan be responsible for a decrease in hardness and an increase in stickiness of cooked rice samples. Ong and Blanshard (1995) reported that the components of leached starch consist of amylose and amylopectin, at a different proportion. Effect of microwave and pressure cooking did not influence on changes of amylose content in cooked-germinated KKU-ULR012 (data not shown). However, rice containing the same amylose content may differ in hardness and stickiness (Juliano and Perez, 1986). Cooking methods (time, temperature, pressure), water to rice ratio and degree of gelatinization have resulted in the hardness and stickiness of cooked rice (Ramesh *et al.*, 2000). Cooked rice contained the moisture content in the range of 64.5 to 74.6% (Bhattacharya, 2000; Ahromrit *et al.*, 2007). Khatoon and Prakash (2006) reported that there was significant difference in the moisture content of cooked rice between cooking methods, such as microwave and pressure cooking, and also between rice varieties. This implied that the varietal differences influenced the water uptake of rice on the application of heat, wherein the microwave cooked sample had less moisture. Ahromrit *et al.* (2007) reported that high pressure cooking induced rapidly water uptake and also facilitated the starch gelatinization of glutinous rice. Furthermore, germination process caused significant changes in the textural characteristics (Komatsuzaki *et al.*, 2007). The data obtained in this study indicate that there was much higher stickiness in germinated KKU-ULR012 after pressure cooking than occurred in the

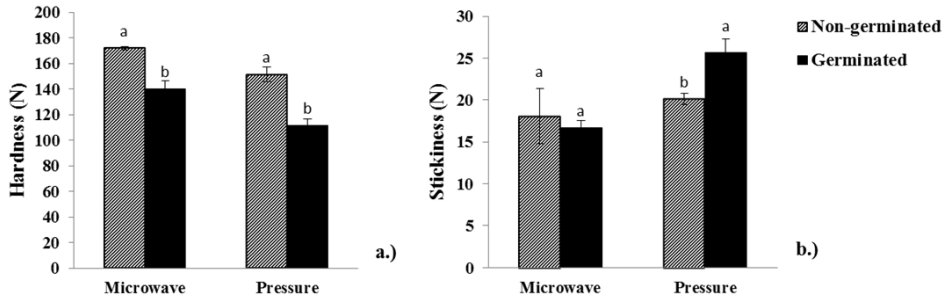

Figure 2. Hardness (a.) and stickiness (b.) values of germinated and non-germinated KKU-ULR012 cooked by microwave and pressure cooking.

grains cooked by microwave, suggesting the pressure cooking is able to improve stickiness in germinated black glutinous rice (KKU-ULR012) (Figure 2b).

3.3 Microstructural characteristics of cooked germinated KKU-ULR012

The microstructures of germinated rice grains uncooked and cooked by microwave and pressure are shown in Figure 3. The individual starch granules of native starches in uncooked germinated KKU-ULR012 are shown in Figure 3a, the starch granules of the uncooked grain showed characteristically irregular polygons. Germination destroys the structure of starch granules. It also introduced more pores in starch granules and lesser compactness, which spread out in the structure due to the action of endogenous enzymes inside grain and decompose large molecular substances, such as starch, non-starch polysaccharides and proteins, to smaller compounds (Moongngarm *et al.*, 2010). Granule morphology of germinated KKU-ULR012 appears to have been affected by both microwave (Figure 3b) and pressure cooking (Figure 3c) that cooked starch granules showed clearly more aggregation than uncooked granules (Figure 3a). This result suggests that changes in starch granular physical structure occurred due to internal recrystallization processes. Damage of starch granules and degree of gelatinization with respect to size, shape or birefringence do not occur during controlled application of heat/moisture to starches, as previously reported by Stute (1992). In Figure 3b and 3c, the grain surface of cooked rice becomes smooth indicating that more starch leached out during cooking. The grains were fully gelatinized. In addition, cooked rice by pressure method showed slightly surface fission at the surface. During heating and high pressuring, rice was changed in the structure of starch, results in gelatinization (Ahromrit *et al.*, 2007). Bilbao-Sáinz *et al.* (2007) reported that both conventional and microwave heating caused starch gelatinization.

4. Conclusion

Cooking methods (pressure and microwave heating) show significant effects on the functional properties of germinated black glutinous rice (KKU-ULR012). The germinated KKU-ULR012 cooked by the pressure method contained higher levels of total anthocyanin, GABA and γ-oryzanol contents and antioxidant activity (DPPH and ABTS) than the germinated samples cooked by the microwave method. Furthermore, the grains cooked by pressure cooking were more moist and sticky than the grains cooked by microwave cooking. Although heating cooked the rice grains for consumption, but it also destroyed bioactive compounds. However, pressure cooking tended to prevent loss of anthocyanin content and antioxidant activity. The data gives an idea of effective process condition to produce cooked-germinated rice which contains GABA, γ-oryzanol content and antioxidant activity for consumption and development of new products at industrial level.

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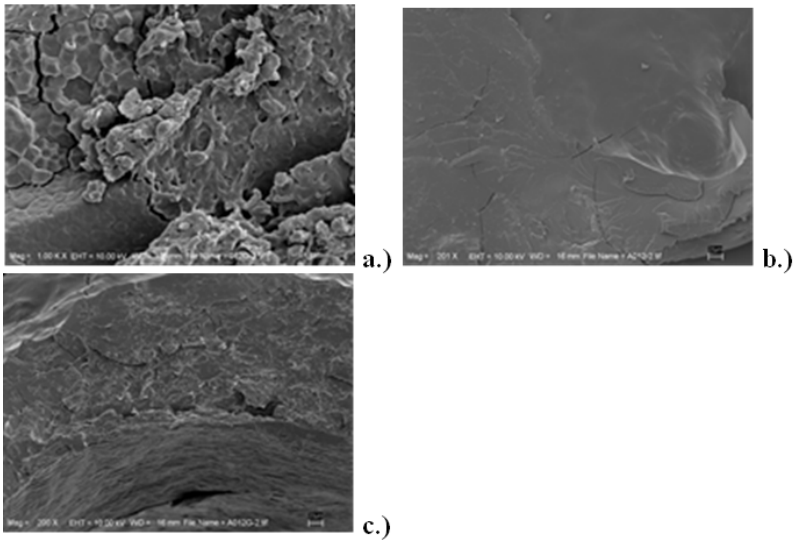

Figure 3. Scanning electron micrographs of uncooked-germinated (a), microwave cooked-germinated (b), and pressure cooked-germinated (c) KKU-ULR012.

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